REMARKS

Status of the Claims

Claims 1-78 are cancelled without prejudice to future prosecution. Claims 79 to 81 are amended. Claims 82 to 99 are new. Therefore, claims 79 - 99 are pending.

Claims 61 and 79 stand objected to for various typographical errors. These objections have now been cured by the claim amendments.

All the claims stand rejected on various grounds pursuant to 35 U.S.C. §112: The phrase "an amino acid substantially identical to SEQ ID NO:2" is rejected as representing new matter, failing to comply with the written description requirement. *See* items 10, 11, and 12, respectively of the Official Action mailed December 29, 2005.

Additionally, all the claims stand rejected as allegedly not enabled with respect to the 'linker' subject matter and to the breadth of the "substantially identical" subject matter.

Applicants respectfully reply to these objections and rejections by amendment and argument below.

Status of the Declaration or Oath of Inventor

Applicants provide a copy of the Declaration or Oath as signed by the inventor, Andrew Cubitt. Applicants respectfully request the objection be withdrawn.

Status of the Specification

The specification was objected with respect to the priority claim set forth in the first paragraph and the description of Figure 1. The specification was amended to update the priority claim. The description of Figure 1 has been amended to set forth Figures 1A and 1B as shown in the Figures. Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry. As these amendments resolve the concerns identified by the Examiner, Applicants request withdrawal of these objections.

Support for the Amendments to the Claims

Claims 79 to 81 were amended to set forth percent identities of at least 85% over a length of 150 amino acids as disclosed in the paragraph bridging pages 14 and 15 of the specification as filed. Claim 79 was also amended to correct the typographical errors identified by the Examiner.

Claims 79 to 81 were further amended to set forth recitals related to the fluorophore formed from oxidation and cyclization of residues 65 to 67. This subject matter finds support in paragraph 87 of the published specification.

Claims 79 to 81 were further amended to set forth wherein said donor fluorescent protein moiety and said acceptor fluorescent protein moiety exhibit fluorescence resonance energy transfer when said donor fluorescent protein moiety is excited. Support for this recital is found in the first paragraph of the summary of the invention at page 2.

Claims 79 to 81 were further amended to set forth subject matter recited in previous claim 60.

New claims 82 to 84 depend from claims 79 to 81, respectively, and set forth per cent identities described in the paragraph bridging pages 14 and 15 of the specification.

New claims 85 to 90 depend from claims 79 to 84, respectively, and set forth subject matter recited in previous claim 59.

New claims 91 to 96 depend from claims 79 to 84, respectively, and set forth subject matter of recited in previous claim 61.

New claim 97 depends from claim 79 and sets forth at least 85% sequence identities with regard to the whole sequence of SEQ ID NO:2. Support for this subject matter is found in the specification in the paragraph bridging pages 14 and 15.

New claim 98 depends from claim 82 and sets forth at least 95% sequence identities with regard to the whole sequence of SEQ ID NO:2. Support for this subject matter is found in the specification in the paragraph bridging pages 14 and 15.

New claim 99 depends from claim 98 and sets forth a linker of from 12 to 40 amino acids in length. Support for this subject matter is found in the specification in the paragraph bridging pages 20 and 21.

Accordingly, the Applicants believe the amendments to the claims add no new matter and respectfully request their entry.

Response to the new matter rejection based upon the recital of "substantially identical"

Without acquiescing on the merits and in order to expedite prosecution of the application, the Applicants have amended the claims such that the claims no longer recite "substantially identical." Accordingly, the Applicants respectfully request the above rejections to be reconsidered and withdrawn.

Response to the written description rejection as to the recital of "substantially identical"

As amended, each of the independent claims 79 to 81 now to set forth sequence identities of at least 85% over a contiguous stretch of 150 amino acids. Additionally, dependent claims 82 to 84 set forth sequence identities of 95% over 200 contiguous amino acids. And new claims 100 and 101 set forth sequence identities over the full length of SEQ ID NO:2. Further each of independent claims 79 to 80 were amended to set forth essential properties of the acceptor and donor fluorescent proteins related to the oxidation and cyclization of residues at positions 65 to 67 of SEQ ID NO:2 and their ability to undergo FRET.

With regard to claims 82 to 84 which set forth sequence identities of at least 95%, Example 14 of the *Synopsis of Application of Written Description Guidelines* (enclosed) concerns a much more limited disclosure which taught the full sequence of a novel protein, the function of that protein, and how to assay for it. Unlike here, the Example 14 disclosure did not exemplify any variants of the protein and did not introduce any supportive prior art teachings. Yet, USPTO guidelines found the disclosure provided adequate written support for claims reciting 95% sequence identity. Accordingly, as to claims 82 and 84, the Applicants request the above rejection be reconsidered and withdrawn.

Applicants next address the grounds for the rejection of claims 79 to 81 which set forth sequence identities of at least 85%. The specification does comply with current patent law

and practice regarding description of a nucleic acid or amino acid sequence. As noted by the Examiner, the Federal Circuit court of Appeals addressed the description adequate to show one of skill that the inventors were in possession of a claimed genus at the time of filing. *See, e.g., Enzo Biochem, Inc. v. Gen-Probe, Inc.*, 63 USPQ2d 1609 (Fed. Cir. 2002). An applicant may also show that an invention is complete by

> . . . disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention . . . *i.e.*, complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics. *Id.* at 1613.

Furthermore, "description of a representative number of species does not require the description to be of such specificity that it would provide individual support for each species that the genus embraces." *See, e.g.*, 66 Fed. Reg. 1099, 1106 (2001). "In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus." *University of California v. Eli Lilly & Co.*, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). M.P.E.P. 2163.

As noted previously, the claims provide both structural and functional limitations for the donor and acceptor fluorescent protein moieties. The structure of the polypeptides is recited as a formula, in keeping with the Federal Circuit decision of *Lilly*. Moreover, the specification *incorporates by reference* several pertinent publications in the paragraph bridging pages 14 and 15. These publications disclose a great many embodiments of suitable acceptor and donor fluorescent protein moieties which can be modified for use according to the instant specification: See, enclosed PCT/US95/14692, filed Oct. 10, 1995 (enclosed U.S. Patent No. 5,777,079 which matured from the corresponding National Stage application), and enclosed U.S. Patent Application No. 6,124,128 which matured from the incorporated U.S. Patent Application

Serial No. 08/706,408. In particular, these references teach the critical fluorophore region of SEQ ID NO:2 and many useful mutations in that region as well as in other regions.

In *Falkner v. Inglis*, No. 05-1324 (Fed. Cir. May 26, 2006) the Federal Circuit ruled that, for claims to nucleic acid sequences and by analogy to amino acid sequences, absence of examples does not render written description inadequate and that actual reduction to practice is not required. *See, e.g., Falkner* slip op at page 14. The court also ruled that publicly available references that describe essential regions of a pox virus could be used to allow those of skill to choose an essential vaccinia gene and then to make a claimed virus. *See, e.g., Falkner* slip op at page 13. In the present case, the specification directly teaches or incorporates by reference teachings as to the functionally important regions of SEQ ID NO:2 to assist those of skill in designing functional polypeptides with at least 85% identity as recited in the independent claims. Further, Heim *et al.*, PNAS 91 (26): 12501 (1994) made a great many mutations while focusing on the more interesting ones which changed the fluorescence properties of the protein. Regardless, Heim *et al.* evidences how easily suitable mutants of green fluorescent proteins may be made and identified.

There is no *per se* bar as to sequence identities of at least 85% sequence identity. The Applicants further invite the Examiner's attention to the *Sun* decision by the Board of Patent Appeals and Interferences (see, *Ex parte Sun*, Appeal No. 2003-1993 on Application No. 09/470,526, enclosed). In *Sun,* the Board considered a claim to a polynucleotide sequence having 80% identity to the entire coding region of a disclosed polynucleotide sequence. The *Sun* Applicants had not disclosed a single representative species with 80% identity and the recited function. The Examiner in *Sun* had argued that the specification did not teach a single variant with the pertinent function. The Board did not find the fact that the specification does not specifically teach the structure of a species with 80% identity and the WEE1 function to be dispositive of the written description question. Rather, the Board looked to the teachings of the prior art and concluded in view of those teachings which identified critical portions of the protein that one of ordinary skill in the art would have recognized that the Applicants were in possession of the claimed subject matter. As discussed immediately above, the Applicants disclosure sets

forth just such supplemental information either expressly or by incorporation of references that do.

In summation, the Applicants' disclosure provides written description support far exceeding that set forth in Example 14 of the Written Description Guidelines. Accordingly, the Applicants merit claims of correspondingly greater scope and respectfully request that the above rejection be reconsidered and withdrawn.

Enablement of the "substantially identical" and linker subject matter.

As noted by the Examiner, whether undue experimentation is required to practice an invention is typically determined by the *Forman* factors. These factors weigh (i) the relative skill of those in the art; (ii) the nature of the invention; (iii) the breadth of the claims; (iv) the amount of guidance presented; (v) the presence of working examples; (vi) the state of the art; (vii) the predictability of the art; and (viii) the quantity of experimentation necessary. *Ex parte Forman*, 230 U.S.P.Q. 546 (PTO Bd. Pat. App. & Inter. 1986), *In re Wands*, 858 F.2d 731, 8 U.S.P.Q.2d 1400 (Fed. Cir. 1988). The Applicant first reviews the claims at issue and then addresses each of these factors, particularly as raised by the Examiner, and thereafter provides a general summary.

The Claims

The rejected methods claims have been amended in order to expedite prosecution of the application without acquiescing on the merits to a position adopted by the Examiner. Independent claims 79 to 81, as amended, now set forth sequence identities of at least 85% over a contiguous stretch of 150 amino acids.

(i) Level of Skill in the Art.

Applicants believe that the relative skill and experience of those in the art of protein engineering is very high. Such work is typically conducted by research enterprises populated with persons with advanced degrees and extensive training" in the relevant fields. Support for this assertion is readily evidenced by the authorship of the art disclosed with the previous IDS and discussed herein.

(ii) Nature of the Invention.

The invention is in the field of protein engineering. More particularly the invention is in the field of modified green fluorescent proteins. Additionally, the invention lies in the field of the physical chemistry with regard to fluorescence resonance energy transfer. It is a field in which the measure of activity is particularly easy to monitor and the art of record has evidenced the ease with which the protein moieties can be reengineered (see, Cubitt et al., *Trends in Biochemical Sciences* 20(11):448 455 (1995), already of record).

(iii) Breadth of the Claims.

Without acquiescing on the merits, and in order to expedite prosecution of the Application, Applicants have amended the independent claims as discussed above to set forth sequence identities of at least 85% for the respective fluorescent protein moieties. Dependent claims 82 to 84 set forth sequence identities of at least 95%. The claims further required that the residues at positions 65 to 67 be oxidized and cyclized to form the fluorophore.

(iv) Amount of Guidance Presented.

The specification provides adequate guidance for all manipulations required to practice the invention. As discussed above, the Applicants' disclosure expressly or by incorporated reference discloses the important portions of the acceptor and donor fluorescent protein moieties and how they may be modified and then combined into constructs of the invention. In this regard, Applicants note that incorporation by reference is no bar to enablement. The MPEP § 2163.07(b) provides:

> [The] Commissioner has considerable discretion to permit the applicant to incorporate information by reference into the specification. The information incorporated by reference at the time of filing is as much a part of the application as filed as if the text were repeated therein.

The Applicants' disclosure teaches how donor and acceptor pairs are selected according to an overlap in the donor emission and acceptor excitation wavelengths (see paragraphs 49 and 39) and coupled. Wavelength values are readily obtained using a fluorometer and many are set forth in PCT/US95/14692 which was incorporated by reference. Table 1 at

page 16 sets forth many such values. The Applicants' disclosure also sets forth all the necessary technical guidance for coupling the donor and acceptor moieties and for making and screening resulting tandem constructs. The disclosure also sets forth on pages 19 and 20 both general principles and particular mutations which would further provide a variety of possible ancillary mutations to enhance a construct.

(v) Working Examples.

Using a linker moiety including cleavage recognition sites for many proteases, the specification disclosed how to make and use five tandem constructs according to the invention Examples 1 and 2 (starting at page 40). These proteases included trypsin, enterokinase and calpain. For instance, the first described construct had a donor fluorescent protein moiety having a S65C mutation linked to an acceptor fluorescent protein having a Y66H/Y145F mutation. Example 2 appears also to have looked at a sixth tandem construct having the W1B donor moiety and a 10c acceptor moiety.

The specification also exemplified at page 21 another 8 constructs based upon donor acceptor pair comprising the Sapphire donor protein moiety and the Topaz acceptor fluorescent protein moiety. These constructs varied by the length of their linker moiety. These linkers varied from 12 to 25 amino acids in length

Accordingly, the specification evidences the ability to separately modify the acceptor and donor fluorescent protein moieties and then integrate them into a number of useful constructs.

(vi) State of the Art.

The state of the art is high. The methods for generating fluorescent protein moieties for use according to the invention are exemplified by the Heim et al. reference. Suitable moieties are found in the art and particularly as incorporated by reference and discussed above. Using well known methods and moieties previously disclosed in such references, the Applicants were able to select a substantial number of acceptor and donor fluorescent moieties to make the claimed constructs. This state of the art, when coupled to the Applicant's disclosure,

provides one of ordinary skill in the art with the information, moieties, and tools needed to broadly practice the invention as claimed.

(vii) Unpredictability of the Art.

The field of protein structure and function is generally one in which unpredictability can be a concern. However, green fluorescent proteins are amongst the most studied, characterized and reengineered of proteins (see, IDS setting forth a number of patents in this field). The Applicants have cited the Heim et al. reference which taught the critical portion of the fluorescent green protein and incorporated by reference others (e.g., PCT/US95/14692) which expanded upon those teachings and evidenced many useful modified green fluorescent proteins of SEQ ID NO:2 that are already available. Accordingly, the Applicants submit that the field of art pertinent to the present claims is mature and very well developed.

(viii) Undue Experimentation.

The quantity of experimentation necessary[1] to practice the invention with exemplified and non-exemplified embodiments is what is routinely performed by a person of ordinary skill in the art as illustrated in the instant specification and, for instance, recognized in U.S. Patent No. 6,803,188, U.S. Patent No. 5,777,079, and U.S. Patent No. 5,981,200. Indeed, in demonstrating their ability to make and use six tandem constructs, the Applicants have shown it is possible to readily identify active constructs with an amount of research which is no more than that representative of the field. The relative ease by which suitable mutants for the fluorescent protein moieties of the constructs can be obtained (e.g., using random mutagenesis as taught by the Applicants and by the Heim) greatly reduce the experimental effort required to obtain constructs according to the invention.

[1] That some experimentation may be necessary to identify operative species does not constitute a lack of enablement. As the Federal Circuit has stated, "the key word is 'undue', not 'experimentation' " in determining whether pending claims are enabled. *Wands*, 8 U.S.P.Q.2d at 1405 (Fed. Cir. 1988). Indeed, a considerable amount of experimentation is permissible if it is merely routine, or if the specification in question provides a reasonable amount of guidance for practicing the invention.

(ix) Summary and Overall *Forman/Wands* Analysis.

As set forth in the MPEP §2164.01(a), the final step in making the determination that "undue experimentation" would have been needed to make and use the claimed invention is reached by weighing all the above noted factual considerations. *In re Wands,* 858 F.2d at 737, 8 USPQ2d at 737."

Here, the level of skill for one of ordinary skill in the art is high. The breadth of the methods claims at issue is more clearly focused as the Applicants have amended the claims to set forth subject matter related to the method of operation of the invention. Furthermore, the invention is in a field of art which is highly advanced and in which considerable screening is undertaken as a matter of routine. In addition, the field has is mature and has available to it combinatorial chemistry and high throughput screening methodologies to facilitate such screening on a large scale. In addition, the power of the research techniques available to one of ordinary skill in the art overwhelms any uncertainty in the art by permitting a vast number of suitable fluorescent protein moieties to be made and screened with ease. Thus, one of ordinary skill in the art would need to do no more than the routine amount of experimentation in order to practice the invention as presently claimed.

In light of the above Remarks and Amendments, Applicants believe that one of ordinary skill in the art can practice the invention as presently claimed according to the requirements of 35 U.S.C. §112, 1st paragraph. Accordingly, Applicants respectfully request that the above rejection be reconsidered and withdrawn.

Enablement of the Linker Subject Matter

Applicants note, as a threshold matter, that each of independent claims 79 to 81 have been amended to set forth the donor moiety acceptor moiety and the linker moiety are fused in a single amino acid sequence.

The claims stand rejected particularly with regard to an alleged lack of enablement with regard to the "linker moiety." More specifically, the Action contended that the term "linker moiety" includes potentially inoperative embodiments, such as "linkers that are

fluorescent," or linkers that are "too short" possibly resulting in steric clashes between the donor and acceptor moieties or the inability of a cleavage enzyme to attack the linker. Applicants respectfully disagree.

Applicants respectfully note that "[t]he presence of inoperative embodiments does not necessarily render a claim nonenabled." See MPEP §2146.089(b). Indeed, the Federal Circuit has held that "[t]he standard is whether a skilled person could determine which embodiments that were conceived, yet not made, would be inoperative or operative with expenditure of no more effort that is normally required in the art." *See Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984).

Here, Applicants' specification clearly identifies the characteristics of operative embodiments and facile experiments to determine operability. For example, the Summary of the Invention clearly states that "the donor and acceptor moieties exhibit fluorescence resonance energy transfer when the donor is excited." See page 2, lines 15-30. Thus, one skilled in the art would immediately understand the desirability of a linker moiety that does not interfere with the fluorescence resonance energy transfer between the donor and acceptor. Using basic principles of fluorescence widely known in the art, one skilled in the art would select linker moieties that do not substantially fluoresce or absorb fluorescence. When in doubt, one skilled in the art would perform a simple assay as described on page 21 of Applicants' specification to assess the effect of the linker "on the ability of tandemy linked fluorescent proteins to become fluorescent." See page 21,lines 10-30.

With respect to length, Applicants' specification clearly states that "[t]he length of the linker moiety is chosen to optimize both FRET and the kinetics and specificity of enzymatic cleavage." Thus, one skilled in the art would immediately understand that the length of the linker moiety would not be selected at random. Rather, the known dependency of FRET upon distance should be taken into account. Indeed, Applicants' even describe the known mathematical parameters of FRET as a convenience to those of skill in choosing an applicable linker moiety length. (See, page 17, line 29 to page 19, line 1). In fact, Applicants' even go to the trouble of suggesting certain specific linker moiety lengths (see , page 20, line 8 to page 21,

line 29 and sequences of Table III on page 24) as additional guidance to those skilled in the art. With regard to the unpredictability of protease accessibility and cleavage of the linkers, Example 2 provides data showing that one of ordinary skill can readily engineer cleavage sites which are robust even as to having multiple cleavage sites which are accessible to any of three exemplified enzymes (e.g., calpain, enterokinase, trypsin). This results agree with the well known status of such protease cleavable linkers in the related field of bioconjugation.

In light of the extensive guidance provided by Applicants' specification in the selection of linker moieties and the knowledge in the art, it cannot be fairly asserted that a skilled person would have trouble determining which linker moieties would be operative.

Accordingly, the Applicants believe that one of ordinary skill in the art can practice the invention as presently claimed according to the requirements of 35 U.S.C. §112, 1st paragraph. Applicants respectfully request that the above rejection be reconsidered and withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested.

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at (925) 472-5000.

Respectfully submitted,

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